

## WE CLAIM:

1. A method comprising
  - (a) identifying a non-nucleotide prototype compound;
  - 5 (b) substituting the prototype compound with a phosphonate-containing group to produce a candidate compound; and
  - (a) determining the anti-HIV activity of the candidate compound.
2. A method comprising
  - 10 (a) selecting a non-nucleotide candidate compound containing at least one esterified carboxyl or esterified phosphonate-containing group; and
  - (b) determining the intracellular persistence of the candidate compound or a esterolytic metabolite of the esterified carboxyl or phosphonate-containing group thereof.
3. The method of claim 1 wherein the tissue selectivity of the candidate compound  
15 and/or at least one of its intracellular depot metabolites is determined.
4. The method of claim 1 wherein the intracellular residence time of said candidate compound and/or at least one of its intracellular depot metabolites is determined.
5. The method of claim 2 comprising additionally determining the activity of at least one of said metabolites against HIV protease.
- 20 6. The method of claim 2 wherein the metabolite is a carboxylic acid.
7. The method of claims 1 or 2 comprising determining the ability of the candidate to inhibit HIV.
8. The method of claim 1 wherein the prototype is already known to have therapeutic activity against HIV.
- 25 9. The method of claim 2 comprising selecting and determining the intracellular persistence of a plurality of candidate compounds.

10. The method of claims 1 or 2 wherein compounds which are not candidate compounds are tested in parallel together with at least one candidate compound.

11. The method of claim 2 comprising determining cleavage of one or more candidates by GS-7340 Ester Hydrolase.

5 12. The method of claims 1 or 2 wherein the candidate is an amino acid phosphonoamidate in which a carboxyl of the amino acid is esterified.

13. The method of claim 1 wherein the prototype compound is known to inhibit HIV protease, HIV integrase or HIV reverse transcriptase.

10 14. The method of claim 1 wherein the prototype compound is not known to be an analogue of a naturally occurring phosphate-containing enzyme substrate.

15. The method of claim 1 wherein the prototype compound is not a nucleoside.

16. The method of claim 1 wherein the prototype compound does not contain a nucleoside base.

17. The method of claim 1 wherein an intracellular depot metabolite is tested.

15 18. The method of claim 1 also comprising determining the resistance of HIV to the candidate compound and/or its intracellular depot metabolite.

20 19. The method of claim 1 comprising determining the tissue selectivity and/or intracellular residence time for a first candidate compound and/or its intracellular depot metabolite, preparing or selecting additional analogues of said first candidate compound, and determining the therapeutic activity of said additional analogues without determining tissue selectivity and/or intracellular residence time of said analogues.

20. The method of claim 1 comprising determining the safety and/or anti-HIV therapeutic activity of the candidate compound in *in vitro* cell culture, in enzyme assay, in animals or in humans.

25 21. The method of claim 1 wherein the prototype compound is a pharmaceutical product licensed by the US Food and Drug Administration.

22. The method of claim 1 wherein the prototype compound is one which is disclosed to have anti-HIV activity in a patent or published patent application on or before the filing date of this application.

23. The method of claim 1 comprising determining susceptibility to hydrolysis of the carboxyl or phosphonate esters by GS-7340 Ester Hydrolase, said Hydrolase characterized by being capable of being recovered from human PBMCs by a process comprising

(b) lysing human PBMCs;

(c) extracting the lysed cells with detergent;

(d) separating the solids from supernatant and recovering the supernatant;

10 (e) contacting the supernatant with an anion exchange medium;

(f) eluting the Hydrolase from the anion exchange medium;

(g) contacting the eluate with a hydrophobic chromatographic medium; and

(h) eluting the Hydrolase from the hydrophobic chromatographic medium.

24. The method of claim 23 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa, has a pI of about 4.5-5.5 by chromatofocusing, is inhibited by 3,4 dichloroisocoumarin, binds to Butyl Sepharose HIC, binds to anion exchange medium Q15, and is capable of being recovered from human PBMCs.

25. The method of claim 2 wherein the intracellular residence time is determined as the half-life of at least one intracellular depot metabolite within a lymphoid tissue.

20 26. The method of claim 25 wherein the lymphoid tissue is PBMCs, helper cells, killer cells or lymph nodes.

27. The method of claim 1 wherein determining anti-HIV activity is by *in vitro* assay.

28. The method of claim 27 wherein the assay is conducted in an animal model or clinical trials.

25 29. The method of claims 1 or 2 comprising the additional steps of identifying a clinical trial compound from the final step, entering into clinical trials with said clinical trial

compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.

30. The method of claim 29 wherein the clinical trial compound is not identical to the candidate compound

5 31. The method of claim 2 wherein intracellular persistence was determined by clinical studies comprising determination of the amount and timing of dosing of the candidate compound.

32. The method of claim 2 wherein the metabolite is intracellularly sequestered in PBMCs.

10 33. The method of claim 2 wherein greater than one metabolite is tested to determine intracellular residence time.

34. The method of claim 2 wherein the intracellular persistence is determined in PBMCs.

15 35. The method of claim 2 wherein the metabolite comprises the phosphonate group of Metabolite X.

36. The method of claim 2 wherein the metabolite comprises an unesterified carboxyl group.

37. The method of claim 2 wherein the intracellular depot metabolite comprises the group  $-P(O)(OH)-$ .

20 38. A library of candidate non-nucleotide anti-HIV compounds comprising a plurality of candidate compounds suspected to have anti HIV activity which contain esterified carboxyl or esterified phosphonate groups.

25 39. A library of candidate anti-HIV compounds which does not consist solely of nucleotides and which comprises a plurality of candidate compounds suspected to have anti-HIV activity which contain esterified carboxyl or esterified phosphonate groups.

40. The library of claims 38 or 39 comprising at least about 10 candidate compounds.

41. The library of claims 38 or 39 wherein the candidate compounds comprise (a) a phosphonate substituted with an amino acid or an organic acid, or (b) an amino acid, at least one of the carboxyl groups of the amino acid or organic acid being esterified.

5 42. The library of claims 38 or 39 wherein the compounds in the library are stored in discrete containers.

43. A method comprising testing the library of claims 39, 40, 41, or 42 to determine the anti-HIV activity of at least one candidate compound in the library.

10 44. The method of claim 43 comprising determining for tissue selectivity and/or the intracellular persistence of at least one of said candidate compounds and/or at least one of their intracellular metabolites.

45. The method of claim 43 comprising the additional steps of identifying a clinical trial compound from said library, entering into clinical trials with said clinical trial compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.

15 46. Isolated GS-7340 Ester Hydrolase.

47. The Hydrolase of claim 46 which is purified to a single major band on gel filtration chromatography.

48. The Hydrolase of claim 46 which is capable of being recovered from human PBMC cells.

20 49. The Hydrolase of claim 48 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa.

50. The Hydrolase of claim 50 which has a pI of about 4.5-5.5 by chromatofocusing

51. The Hydrolase of claim 50 which is inhibited by 3,4 dichloroisocoumarin,

52. The Hydrolase of claim 51 which binds to Butyl Sepharose HIC.

25 53. The Hydrolase of claim 52 which binds to anion exchange medium Q15.

54. The Hydrolase of claim 53 which binds to hydroxyapatite.

55. The Hydrolase of claim 46 which is cross-linked to an insoluble medium.

56. A method comprising obtaining a substantially pure organic molecule, optionally contacting the organic molecule with another molecule to produce a composition, and contacting the Hydrolase of claim 46 with said organic molecule or composition.

5 57. The method of claim 56 wherein the organic molecule is an anti-HIV compound.

58. A method comprising contacting GS-7340 Ester Hydrolase with an organic compound in an *in vitro* or cell culture environment.

59. The method of claim 58 wherein the environment is cell free.

10 60. A composition comprising a substantially pure organic compound and isolated GS-7340 Ester Hydrolase.

61. A composition comprising an organic compound and GS-7340 Ester Hydrolase in an *in vitro* or cell culture environment.

15 62. In a method for identifying an anti-HIV therapeutic compound, the improvement comprising substituting a prototype compound with an esterified phosphonate or esterified carboxyl group to produce a candidate compound and assaying the resulting candidate compound for its anti-HIV activity.

63. The method of claim 61 wherein the candidate is assayed for its intracellular persistence.

20 64. The method of claim 63 wherein the candidate is assayed for its extracellular stability against hydrolysis of the carboxyl or phosphonate ester.

65. The method of claim 64 comprising selecting from a plurality of candidates a candidate which is esterolytically cleaved intracellularly to yield an intracellular persistent metabolite having anti-HIV activity and which candidate is substantially esterolytically stable against extracellular hydrolysis of the carboxyl or phosphonate ester.

25 66. The method of claim 65 wherein the candidate is substantially stable against hydrolysis of the carboxyl or phosphonate esters outside of lymphoid tissue.

67. The method of claim 62 wherein the candidate is substituted with a phosphonate group comprising monosubstitution with (a) an amino acid linked through an amino group to the phosphorus atom or (b) an organic acid, and wherein a carboxylic acid of the amino acid or organic acid is esterified.

5           68. The method of claim 62 wherein the candidate is substituted with a group comprising an amino acid, wherein a carboxylic acid of the amino acid is esterified.

69. The method of claim 68 wherein the carboxylic acid is the residue of a hydroxyorganic acid linked to the phosphorus atom through an oxygen atom.

10           70. The method of claims 68 or 69 wherein the hydroxy group of the hydroxyorganic acid or the amino group of the amino acid are in the alpha position.

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